The Effect of Repeated Doses of Intermittent Ketamine on Erythrocyte Deformability in Infant Rats

Tekrarlayan Dozlarda Aralıklı Ketamin Uygulamasının Infant Ratlarda Eritrosit Deformabilitesi Üzerine Etkisi

Hülya Kaşıkara[1], Faruk Metin Çomu[2], Ayşegül Küçük[3], Levent Öztürk[4], Mustafa Arslan[5]

[1]Ankara City Hospital, Anesthesiology and Reamination Clinic, Ankara, Turkey
[2]Kırıkkale University, Medical Faculty, Department of Physiology, Kırıkkale, Turkey
[3]Kütahya Health Science University, Medical Faculty, Department of Physiology, Kütahya, Turkey
[4]Yıldırım Bayazıt University, Medical Faculty, Department of Anesthesiology and Reamination, Ankara, Turkey
[5]Gazi University, Medical Faculty, Department of Anesthesiology and Reamination, Ankara, Turkey

ABSTRACT

Aim: Blood rheology, which is affected by many factors, is also known to be affected by drugs used for anesthesia. Therefore, we aimed to investigate the effects of 21-day ketamine administration on erythrocyte deformability in 3-week-old infant rats
Methods: Twelve 12 week old Wistar Albino male infant rats were randomly divided into two groups. Ketamine group (K), n:6, 50 mg/kg ketamine was administered intraperitoneally at the same time daily for 21 days. Saline group (S), n.6, 50 mg/kg normal saline was administered intraperitoneally at the same time daily for 21 days. After 21 days of administration, all rats were euthanized by intraperitoneal ketamine (100 mg/kg) and taken blood from abdominal aorta. Erythrocytes were obtained from heparinized blood samples. Deformability measurements were performed on erythrocyte suspensions in phosphate buffered saline. For the measurement of erythrocyte deformability, a constant flow filtrometer system was used and the relative resistance was calculated.
Results: Ketamine administration was found to increase relative resistance. The erythrocyte deformability index was significantly higher in the ketamine group compared to the saline group (p:0.006).
Conclusion: We found that long-term repeated ketamine administration negatively affected erythrocyte deformability. In our study, we think that the results obtained in daily anesthesia will be a guide for repeated administration of ketamine, especially in radiation oncology. However, these findings should be supported by clinical and experimental studies in more detailed and large series.

Keywords: Ketamine, repeated administration, infant rat, erythrocyte deformability

ÖZET

Amaç: Birçok faktörden etkilenen kan reolojisini anestezi için kullanılan ilaçların da etkilediği bilinmektedir. Bu sebeple 3 haftalık infant ratlarda 21 günlük ketamin uygulamasının eritrosit deformabilitesi üzerine etkilerini araştırmayı amaçladık.
Yöntem: 3 haftalık 12 Wistar Albino erkek infant ratlar, randomize olarak 2 gruba ayrıldı. Ketamin grubu (K), n=6, 21 gün boyunca hergün aynı saatte intraperitoneal yoldan 50 mg/kg ketamin uygulandı. Salin grubu (S), n=6, 21 gün boyunca her gün aynı saatte intraperitoneal olarak aynı hacimde normal salin uygulandı. 21 günlük uygulamanın sonrasında tüm ratlara intraperitoneal ketamin 100 mg/kg uygulanarak abdominal aortadan kan alınarak ötenazi uygulandı. Eritrositler heparinize tam kan örneklerinden elde edildi. Deformabilite ölçümleri fosfatla tamponlanmış serum fizyolojik içerisindeki eritrosit süspansiyonlarında yapıldı. Eritrosit deformabilitesi ölçümü için sabit akımlı filtrometre sistemi kullanıldı ve rölatif resiztans hesaplandı.
Bulgular: Ketamin uygulamasının rölatif rezistansı arttırdığı bulundu. Ketamin uygulanan grupta salin uygulanan gruba göre eritrosit deformabilite indeksi anlamlı olarak yüksek tespit edildi (p=0.006).
Sonuç: Literatürde ketaminin infant rat beyin dokusuna etkileri ile ilgili çalışmalar olmasına karşın, infant eritrosit deformabilitesi üzerine etkileri ile ilgili çalışmalara rastlamadık. Uzun süreli tekrarlayan ketamin uygulamasının eritrosit deformabilitesini olumsuz etkilediğini tespit ettik. Çalışmamızda ulaştığımız sonuçlar, günlük anestezi pratiğinde özellikle radyasyon onkolojisinde olmak üzere mükerrer uygulanan ketaminle ilgili yol gösterici olacağını düşünmekteyiz. Ancak bu bulguların daha detaylı ve geniş serilerde yapılacak klinik ve deneysel çalışmalarla desteklenmesi gerekmektedir.

Anahtar Sözcükler: Ketamin, infant rat, eritrosit deformabilitesi

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ORCID IDs: HK: 0000-0002-4312-8560, F.M.Ç:0000-0002-1106-0741, A.K: 0000-0001-9316-9574, L.Ö: 0000-0001-9755-033X, M.A: 0000-0003-4882-5063

Address for Correspondence / Yazışma Adresi: Mustafa Arslan, MD Gazi University Medical Faculty Department of Anesthesiology and Reanimation 06510 Ankara, Turkey
E-mail: mustarslan@gmail.com

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INTRODUCTION

Drugs used during anesthesia may affect not only systemic cardiovascular functions, but also microvascular fluidity with a direct effect on microcirculation and especially hemorrhology. At the same time, hemorrhagic changes can significantly affect the induction and recovery times with anesthetic agents (1).

In anesthesia practice, sedation with ketamine is widely used especially in children. It is the most preferred agent for sedation in children who receive daily radiotherapy for a period of time. Ketamine is an anesthetic in the phencyclidine group and was first used in 1965. It differs from most other induction agents due to its significant analgesic effect. It generally does not depress the cardiovascular and respiratory system. It consists of two stereoisomers: S - (+) and R - (-). The S (+) isomer is more potent and has fewer side effects. Interest in ketamine has recently increased as a result of its effects on hyperalgesia and opioid tolerance. at subanaesthetic doses, it is a relatively selective and potent antagonist of the N-methyl-D-aspartate (NMDA) receptor belongs to the class of uncompetitive antagonists and, when clear, NMDA binds to a specific site within the receptor channel and blocks the receptor. Like other compounds of this class, ketamine can cause hallucinations or other undesirable central effects that limit its clinical use (2).

In recent animal studies, repeated doses of long-term ketamine have been shown to accelerate neuronal apoptosis in the developing rat brain, causing hippocampal neurodegeneration and long-term cognitive impairment (3-5). It is known that general anesthetic agents are also effective on cardiovascular function and microcirculation hemodynamics (1). It is controversial that anesthetic agents alter blood rheology and consequently lead to worsening of tissue perfusion (6). Blood rheology includes hematocrit, plasma viscosity, erythrocyte aggregation and erythrocyte deformability, which have a role in determining the viscosity of blood (7). Previous animal studies have shown that intravenous and inhalation anesthetics affect erythrocyte deformability in young and old rats (8,9). In this study, we aimed to observe the effects of 21 days ketamine administration on erythrocyte deformability in 3-week-old infant rats.

MATERIALS and METHODS

The study was approved by the Ethics Committee of Gazi University Faculty of Medicine (G.Ü.E.T-19.001). Twelve Wistar Albino male infant rats were randomly divided into two groups. Ketamine group (K), (n:6), Saline group (S), (n:6). 21 days. The ketamine group received 50 mg/kg ketamine intraperitoneally every day at the same time and the saline group received normal saline 50 mg/kg intraperitoneally at the same time daily. After 21 days, all rats were euthanized by 100 mg/kg intraperitoneal ketamine for taking the blood from abdominal aorta. Heparinized total blood samples were collected to prepare the erythrocyte suspension. The collected erythrocyte suspensions were used for deformability measurement.

Deformability Measurements

First the samples were centrifuged for ten minutes at 1000 rpm and then serum and the buffy coat on erythrocytes were removed. Then, isotonic PBS buffer was added to the collapsing erythrocytes. This mixture of PBS and erythrocytes was centrifuged for another ten minutes at the same speed of 1000 rpm. Subsequently, liquid was removed from the upper surface. Finally pure red cell packs were obtained from three consequent washing process. PBS buffer was mixed with erythrocyte packs in order to obtain a value. And those mixed suspensions with 5% hematocrit were used for deformability measurement. These procedures were done at 22 ºC.

Deformability parameters were analyzed with the constant-current filtrometer system. Samples of 10 ml erythrocytes suspension - PBS buffer were prepared for measurement. There was a constant flow rate of 1.5 ml/min through an infusion pump. We used a 28 mm nucleoporin polycarbonate filter which has a pore diameter of 5 µm. A transducer detected the pressure changes during the erythrocytes passage through the filter and the collected data was transferred to computer with MP 30 data equation system (Biopac Systems Inc, Commat, USA). The pressure of the system was calibrated before each measurement. Buffer (P_T) and then erythrocytes (P_E) were passed subsequently through from the filtration system and pressure changes were measured. The relative refractory period value (Rrel) was calculated by relating the pressure value of erythrocyte suspension to pressure value of buffer. Increase in Rrel as the deformability index was interpreted as adverse effect on erythrocyte deformability.

Statistical Analysis

SPSS 22.0 software program was used for statistical analyses. The findings were expressed as mean ± standard deviation. Deformability value was evaluated with the Mann-Whitney U test. A $p< 0.05$ was considered statistically significant.

RESULTS

Ketamine was found to increase relative resistance. The erythrocyte deformability index was found to be significantly higher in the ketamine group compared to the saline group (p=0.006), (Figure 1).

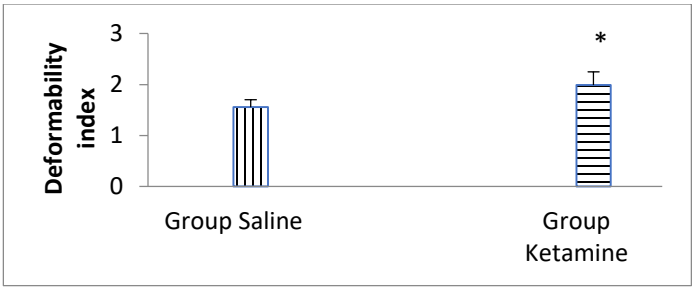

Figure 1: Erythrocyte deformability index values of the groups. Each column represents the Mean ± Standard Deviation * p <0.05; Compared to Saline group

DISCUSSION

Blood exhibits a viscoelastic behavior due to the properties of suspended particles in its content. The most important factor determining the viscosity of the blood in the normal state is erythrocytes. Erythrocytes can be deformed to pass through small vessels. In order for oxygen and vital molecules to be transported to the capillaries and to the final organ, and to remove metabolic waste from this area, erythrocytes must be able to curl, extend and move in these areas. This property is referred to as deformability. Erythrocyte deformability is necessary for normal circulation because erythrocytes should be able to deform when passing through narrow capillaries or when the viscosity of the blood decreases (10).

Erythrocytes as an oxygen carrier; it must continue to fulfill this essential task while being exposed to a wide variety of environments throughout the vascular cycle and various xenobiotics throughout its lifespan. During this time, haeme iron, globin chain and other necessary cellular molecules should prevent continuous oxidant stress (11). Hemolysis, which accelerates the life cycle, occurs following severe and irreversible oxidative damage of aging erythrocytes. One of the most important results of circulating oxidative damage in metabolism due to various reasons is the deterioration of the deformability properties of erythrocytes, which have a very important role in regulation of blood flow and tissue perfusion (10). The deterioration of the rheological parameters of the blood causes the tissue perfusion to weaken.

Anesthesia, surgery duration and surgical intervention itself may affect cell functions and cause free radical formation in metabolism. Free radicals may contribute to posttraumatic and postoperative disorders by targeting the biomolecules of cells such as lipids, carbohydrates, proteins and DNA. Oxidative damages occur when the balance and antioxidant defense system in free radical production is disrupted (12). Lipid peroxidation products caused by oxidative stress damage the membrane permeability and micro viscosity. Thus, a decrease in deformation capacity and survival time of erythrocytes is observed (13).

Ketamine is often associated with variable mitochondrial function and oxidative stress. In the literature, it has been reported that free radicals increase with the use of certain doses of ketamine and that ganglia are exposed to oxidative damage in basal ganglia containing more catecholamine (14-16). De oliveira et al. In an experimental animal model performed by sub anesthetic doses of ketamine to induce a schizophrenia-like condition in the rat brain, it was shown that lipid peroxidation and tissue protein oxidation increased in multiple cerebral structures (17).

Drugs used during anesthesia may affect not only systemic cardiovascular functions, but also microvascular fluidity with a direct effect on microcirculation and especially hemorrhology. Arslan et al.

In this study, the effect of propofol on erythrocyte deformability in male and female rats was investigated. Increased relative resistance, which is a negative indicator of erythrocyte deformability. Therefore, it was concluded that propofol adversely affects erythrocyte deformability (8). Yerer et al. in this study, the effect of desflurane on erythrocyte deformability in old and young rats was investigated. It has been observed that both old and young rats adversely affect deformability (18). Aydogan et al. concluded that sevoflurane reduced erythrocyte deformability in elderly rats and thus negatively affected sevoflurane (19). Gul et al. in their clinical study to observe the effects of sevoflurane and desflurane on plasma and blood viscosity, they stated that sevoflurane had a negative effect on blood viscosity, and that desflurane had a stable effect on the macro circulation in the blood, so that desflurane could be a more preferred anesthetic (9).

In our study, erythrocyte deformability index was found to be high in the ketamine group when compared with the control group. The increase in erythrocyte deformability index is an indicator of the decrease in erythrocyte deformability. This may result in a functional impairment in blood flow and tissue perfusion.

There are too many studies in the literature about the effects of ketamine on infant rat brain tissue, but there is no study about the effects of infant erythrocyte deformability. We found that long-term repeated ketamine administration negatively affected erythrocyte deformability. In our study, we think that the results obtained in daily anesthesia will be a guide for repeated administration of ketamine, especially in radiation oncology. However, these findings should be supported by clinical and experimental studies in more detailed and large series.

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